The relationship between urinary C-Telopeptide fragments of type II collagen, knee joint load, pain, and physical function in individuals with medial knee osteoarthritis

Running title: uCTX-II and knee joint load in subjects with knee osteoarthritis.

Luiz Fernando Approbato Selistre[1], Glaucia Helena Gonçalves[1], Fernando Augusto Vasilceac[1], Paula Regina Mendes da Silva Serrão[1], Theresa Helissa Nakagawa[1], Marina Petrella[1], Richard Keith Jones[2], Stela Márcia Mattiello[1]

[1] Department of Physical Therapy, Universidade Federal de São Carlos (UFSCar), São Carlos, SP, Brazil

[2] School of Health Sciences, University of Salford, Salford, United Kingdom

Corresponding author:

Luiz Fernando Approbato Selistre

Departamento de Fisioterapia, Universidade Federal de São Carlos, Rodovia Washington Luís, Km 235, CEP: 13565-905, São Carlos, SP, Brazil.

E-mail: lfaselistre@gmail.com

Phone: +55 16 3351-9579

Abstract

Objective: Considering the osteoarthritis (OA) model that integrates the biological, mechanical, and structural components of the disease, the present study aimed to investigate the association between urinary C-Telopeptide fragments of type II collagen (uCTX-II), knee joint moments, pain, and physical function in individuals with medial knee OA. **Methods:** Twenty-five subjects radiographically diagnosed with knee OA were recruited. Participants were evaluated through three-dimensional gait analysis, uCTX-II level, the WOMAC pain and physical function scores, and the 40m walk test. The association between these variables was investigated using Pearson´s product-moment correlation, followed by a hierarchical linear regression, controlled by OA severity and body mass index (BMI). **Results:** No relationship was found between uCTX-II level and knee moments. A significant correlation between uCTX-II level and pain, physical function, and the 40m walk test was found. The hierarchical linear regression controlling for OA severity and BMI showed that uCTX-II level explained 9% of the WOMAC pain score, 27% of the WOMAC physical function score, and 7% of the 40m walk test. **Conclusion:** Greater uCTX-II level is associated with higher pain and reduced physical function and 40m walk test performance in individuals with medial knee OA.

Keywords: physical therapy; gait; biomarkers; walk test; disability evaluation.

Highlights

- There is no association between uCTX-II and the knee joint load;
- The uCTX-II level is associated with pain and physical function;
- Knee joint load showed no association with pain and physical function.

Introduction

Knee osteoarthritis (OA) is one of the most prevalent diseases in the world [1], characterized by the degradation of articular cartilage. Cartilage degradation is a consequence of the loss of the normal balance between the synthesis and degradation activity of the chondrocytes [2]. The degradation is considered to be a result of mechanical and biological alterations [3-5]. For this reason, studies have investigated how these changes relate to OA symptoms and whether they can predict knee OA onset and progression [6-8].

The unbalanced activity of the chondrocytes and consequent breakdown of articular cartilage can be caused by abnormal or excessive loading in the joint [9-11]. Knee adduction moment (KAM) has been used to measure the distribution of load between medial and lateral compartments of the knee [12-15], more specifically excessive medial compartment loading as this is the most commonly affected compartment [9]. KAM has been associated with pain [16,17], OA severity [5,18], and progression of the disease [8,19]. Knee adduction angular impulse (KAAI), which is the time integral of the KAM curve during stance, has also been used to measure knee load through a combination of the duration and amplitude of KAM [18]. KAAI is also associated with the presence [7], severity [18], pain, and disability [20] in knee OA. More recently, knee flexion moment (KFM) was proposed to improve the measurement of knee load [21], being associated with cartilage thickness in the early stages of the disease [22]. A longitudinal study demonstrated that higher baseline KAM and KFM in individuals with medial knee OA were shown to be associated with reduced knee cartilage thickness at the five-year follow-up [4]. Hence, knee moment variables (KAM, KAAI, and KFM) may be considered appropriate measures of knee joint load.

Some authors consider mechanical alterations responsible for the occurrence of biological alterations, and consequent degradation of articular cartilage, in most cases of knee OA [5,10,11]. The biological alterations of articular cartilage can be identified by

biochemical markers, also called biomarkers [23]. Urinary C-tylopeptide type II collagen (uCTX-II) has been presented as one of the most important OA biomarkers to detect changes in cartilage [23]. The uCTX-II level from a urine sample can measure the systemic concentration of type II collagen, which is the most abundant protein of the cartilage matrix [24,25]. According to BIPED (Burden of disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic) criteria [26], uCTX-II has the ability to diagnose, predict the progression, and identify the severity of the disease [2,27-30], demonstrating also the ability to identify healthy individuals at high risk of developing knee OA[30,31].

Therefore, both biological and mechanical alterations have been shown to be related to the onset or progression of knee OA, however, no clear association has been shown between these components in the current literature. To our knowledge, only one study has investigated the relationship between uCTX-II and knee loads [32], with the authors finding an association between uCTX-II level and KAM and KAAI during walking. However, this association became non-significant after adjusting for disease severity and walking speed. In addition, they did not investigate the association of uCTX-II with KFM nor with pain and physical function. As KFM has been shown to be associated with cartilage thickness in the early stages of the disease[22], its addition could improve the understanding of the potential relationship between uCTX-II and knee joint load.

Only a few studies have explored the relationship between biomarkers [33] and knee load [34], with pain and physical function. As OA is a persistent condition, current treatments target pain and physical function improvement/maintenance [3,7,19,35,36]. Exploring how mechanical and biological alterations influence these parameters can bring new perspectives for pain and disability control and treatment strategies.

Therefore, the aim of this study was to investigate the association between uCTX-II, knee joint moments (KAM, KFM, and KAAI), pain, and physical function in individuals with medial knee OA. We hypothesized that uCTX-II level is associated with pain, physical function, and knee joint moments (KAM, KFM, and KAAI).

Material and Methods

Design

A cross-sectional design was used.

Sample size

A priori sample size calculation was performed by using G* Power 3.1. The calculation aimed to reach a statistical significance level of 0.05, power of 80%, and a medium effect size (d = 0.5), considering a correlation test and one tail. Based on these parameters, our sample size calculation estimated the need for at least 21 subjects.

Subjects

Community-based volunteers were recruited through advertisements in local newspapers, university websites, and social media. All volunteers underwent anteroposterior semiflexed weight-bearing, lateral view, and skyline view radiographs and were then classified according to the Kellgren and Lawrence (KL) criteria [37]. As the medial knee compartment is the most commonly affected [38], only individuals with predominantly medial knee OA and medial knee pain were included. Therefore, potential participants were excluded if they presented KL grades in the lateral or patellofemoral compartment greater than the medial compartment [39]. In addition, potential participants were excluded for any of the following criteria: body mass index (BMI) greater than 35kg/m^2 to reduce soft tissue artifact of marker movement during quantitative gait analysis, unable to walk unaided for at least 10 minutes, history of hip or knee arthroplasty or osteotomy, had undergone knee surgery or other nonpharmacological treatment in the 6 months prior to

124 the study [40]. For participants with bilateral knee OA, the most symptomatic knee was
125 evaluated. All participants provided written informed consent and the present study was
126 approved by the Ethics committee for Human Investigations at the Universidade Federal
127 de São Carlos (UFSCar), São Carlos, SP, Brazil (CAAE: 41716015.0.0000.5504).

128 *Variables*

129 The dependent variable was uCTX-II level, while independent variables were pain,
130 physical function, and variables obtained from three-dimensional gait analysis.

131 *Dependent variable*

132 The uCTX-II level was measured using fasting urine collected in the early morning
133 (within 2 hours of waking), second void, and all samples were stored frozen at -80°C until
134 analysis. The uCTX-II level was determined using an enzyme linked immunosorbent
135 assay (ELISA) based on a monoclonal antibody raised against a linear six amino acid
136 epitope of human type II collagen C telopeptide (Urine CartiLaps®ELISA)[24]. The uCTX-
137 II level was corrected with creatinine concentration (mmol/L) in the sample using an
138 enzymatic colorimetric routine method[41]. For this correction we used the formula:
139 corrected CTX-II Value = 1000xUrine CartiLaps (µg/L)/Creatinine (mmol/L)[42]. The
140 intra- and inter-assay coefficients of variation are ≤7.8% and ≤12.2%, respectively[42]. All
141 analyses were conducted in duplicate and blinded.

142 *Independent variables*

143 Self-reported pain and physical function were measured using The Western Ontario and
144 McMaster Universities Osteoarthritis Index (WOMAC). The WOMAC index is a
145 disease-specific, tri-dimensional, self-administered questionnaire used to assess health
146 status and health outcomes in individuals with knee OA. The WOMAC contains 24
147 questions and consists of three subscales: pain, stiffness, and physical function with five,
148 two, and seventeen questions, respectively. Answers for each of the 24 questions are

149 scored on a five-point Likert scales (none=0, slight=1, moderate=2, severe=3, extreme=4)
150 with total scores ranging from 0 to 96. Higher scores indicate worse disease severity. The
151 WOMAC questionnaire is well recognized for its adequate validity, reliability, and
152 responsiveness for individuals with knee OA [43]. We used the Portuguese version of the
153 WOMAC[44].
154 Objective physical function was measured using the 40m walk test. The 40m walk test
155 was developed to evaluate the ability to walk quickly over short distances, which is an
156 important activity for a good quality of life. This activity is usually limited in individuals
157 with knee OA[45]. Two marks on the ground were placed 10m apart and a cone was placed
158 2 meters beyond each end of the 10m walkway. Participants, wearing comfortable clothes
159 and shoes, were instructed to walk as fast as possible, without running, along the walkway
160 between the two cones, turn around the cone at the end, return, and repeat for a total of
161 40 m. Participants were timed for this test and based on this time, we calculated the speed
162 as suggested by previous studies [45-47]. A previous study[48] found that intra-class correlation
163 coefficient for inter-rater reliability was 0.96 (95% CI 0.93 – 0.98) and standard error of
164 measurement was 0.06 (95% CI 0.05 – 0.08). The same study[48] found that intra-rater
165 reliability was 0.92 (95% CI 0.82 – 0.96) and the SEM was 0.07 (95% CI 0.06 – 0.09).
166 Three-dimensional gait analysis was performed to measure the KAAI and peak KAM and
167 KFM. Gait was evaluated using an eight-camera Qualisys Oqus 300 motion analysis
168 system (Qualisys, Gothenburg, Sweden) and a force plate (Bertec Corporation, OH, USA)
169 to record kinematic and kinetic data at sampling frequencies of 120 and 1200 Hz,
170 respectively. Participants walked barefoot at a self-selected speed along an 8 m walkway.
171 For each subject, a static calibration trial followed by five successful trials were collected
172 for kinetic and kinematic analysis. The following reflective markers were located on
173 anatomical landmarks bilaterally [49,50]: sternal notch, spinous process of C7, acromion,

iliac crests, anterior and posterior superior iliac spines, greater trochanters of the femur, medial and lateral femoral epicondyles, medial and lateral malleoli, first, second and fifth metatarsal heads, base of the fifth metatarsal, and calcaneus. Four clusters built with 4 noncollinear markers were placed over the lateral side of thighs and shanks. Two additional clusters built with 3 noncollinear markers were positioned on the spinous process of T4 and T12. Markers on the medial and lateral malleoli, femoral epicondyles, C7, greater trochanters, and acromion were removed after the static standing calibration trial was performed. These markers were used to construct the anatomical coordinate system for the trunk, pelvis, thigh, shank, and foot segments.

The ankle and knee joint centers were calculated as midpoints between the malleoli and femoral epicondyles, respectively [51]. The hip joint center was measured using the regression model based on the anterior and posterior superior iliac spine markers [52]. The pelvic coordinate system was built from markers on the anterior and posterior superior iliac spines and then contralateral pelvic drop was measured using a laboratory coordinate system as the reference. The trunk coordinate system was built from markers on the acromion and iliac crest (bilaterally) and the ipsilateral trunk lean was measured using a laboratory coordinate system as the reference. For hip, knee and ankle kinematics we used pelvis, thigh, and shank as local coordinate system respectively. The angular motion of all assessed joints was defined using Cardan angles in accordance with the recommendations of the International Society of Biomechanics [53,54].

The kinetic and kinematic data were processed using Qualisys Track Manager (Qualisys AB) and Visual3D software (C-motion Inc., Rockville, MD, USA). The kinetic and kinematic data were filtered using a fourth-order, zero-lag, low-pass Butterworth filter at cut-off frequencies of 6 and 25 Hz, respectively. Smoothing parameters were set by residual analysis and visual inspection of the processed kinematic and kinetic data.

The stance phase was determined using a force plate, where the initial contact (IC) and toe-off (TO) were identified based on a force threshold of 20N[55]. The kinetic and kinematic data were normalized to 101 points. KFM, KAM, and KAAI were calculated using three-dimensional inverse dynamics[56,57]. KFM and KAM were normalized by the body mass and height (%Bw*Ht), while KAAI was normalized by the body mass, height, and time (%Bw*Ht*s). The peak of each variable throughout the stance phase was used for analysis.

Statistical Analyses

All statistical analyses were performed using SPSS software (Version 20, SPSS Inc., Chicago, IL, USA). The normality of distribution of all variables was analyzed using the Shapiro-Wilk test. As the data presented a normal distribution a Pearson's product-moment correlation coefficient were used to examine the relationship between uCTX-II level, knee moments, symptoms, and physical function. For all significant correlations (uCTX-II with pain, physical function, and the 40m walk test) we processed a hierarchical linear regression. Based on previous studies, we controlled our analysis for OA severity (mild or moderate according to the KL score) [25,58] and BMI (kg/m^2)[59], using these variables as the first step of the hierarchical linear regression. The second step uCTX-II levels was added to the model, which means that all changes in the results of regression analysis (R, R^2, ΔR^2, and p-value), from the first step to the second step, were due to uCTX-II levels inclusion. An alpha level of 0.05 was set for all statistical tests.

Results

A total of 40 potential participants presenting with knee pain were evaluated, however, 15 were excluded: two had a positive test for an anterior cruciate ligament injury, two had significant low back pain (more pain in their back than knee), two presented with hip pain, and the other nine presented with other knee compartments as or

more affected than the medial knee compartment (7 for the patellofemoral joint and 2 for the lateral knee compartment). Twenty-five subjects with knee OA were eligible for the study. For diagnosis, we considered the clinical, radiographic, and history criteria of the American College of Rheumatology [60]. Group characteristics and descriptive values are presented in table 1. A significant correlation between uCTX-II level and pain, physical function, 40m walk test, and gait speed was found (Table 2 and Figure 1) while no significant correlation was found with the other measures.

"INSERT TABLE 1 NEAR HERE"

"INSERT TABLE 2 NEAR HERE"

"INSERT FIGURE 1 NEAR HERE"

After controlling for severity and BMI through a hierarchical linear regression we found that severity and BMI explained 35% of the variance of the WOMAC pain score, while uCTX-II level explained an additional 9% of this variance (Table 3). In addition, severity and BMI explained 39% of the variance in the 40m walk test, while uCTX-II level explained an additional 7% of this variance (Table 3). Finally, uCTX-II level explained 27% of the variance in the WOMAC Physical Function Score (Table 3).

"INSERT TABLE 3 NEAR HERE"

Discussion

This cross-sectional study provides evidence that uCTX-II level is positively associated with pain (r=0.49) and physical function (r=0.53), but negatively associated with the 40m walk test (r=-0.48), even after controlling for OA severity and BMI.

One objective of this study was to investigate the association between uCTX-II level and knee joint moments. Although these variables are related to the onset and progression of the disease, our study could not confirm this association. An earlier study[32] has reported an association of uCTX-II level with KAM and KAAI, however, when

disease severity and walking speed were controlled for in the analysis the association was no longer significant. The present study investigated this relationship not only using the KAM and KAAI, but also KFM as an important measure to improve the ability to measure the medial knee load [21]. There are possible reasons why we did not find an association between uCTX-II and knee joint moments. First, although we used three parameters of medial knee load (KAM, KFM, and KAAI), they do not represent the total knee load. However, as we included subjects with predominantly medial KOA as it is the most commonly affected compartment, the medial knee load was the focus of our analysis. Second, we measured the fasting level of uCTX-II through a sample of the second void of morning urine, which means that our volunteers had limited physical effort in the hours prior to the sample collection. This may have influenced our findings given that the biomarker response to a mechanical stimulus has been shown to be more sensitive to understand the relationship between cartilage metabolism and knee load than only resting levels [61,62]. For this reason, future studies should explore the stimulus-response approach to better understand the relationship between uCTX-II level and knee joint load. Third, although uCTX-II has been used to analyze individuals with knee OA, perhaps uCTX-II level was not sensitive enough to correlate with medial knee load measures because of its systemic characteristics. For this reason, future studies may consider using synovial fluid from the knee to investigate this relationship, as it would provide responses specifically from the cartilage of the knee.

The present study showed that uCTX-II level explained part of the variance in WOMAC pain score (9%), WOMAC physical function score (27%), and the 40m walk test (7%). In addition, the influence of BMI and disease severity were controlled as both measures explained 35% of the WOMAC pain score and 39% of the variance in the 40m walk test. In contrast to these findings, Garnero et al.[33] found no correlation of uCTX-II

levels with the WOMAC total score or subscales (pain, stiffness, and physical function). However, Garnero's et al.[33] study did not control the influence of BMI and disease severity which may have influenced their results.

Taking into account that uCTX-II levels represent cartilage destruction, and considering that this is one of the factors influencing knee pain in individuals with knee OA [63], finding a variation of 9% in WOMAC pain score assigned to the uCTX-II level is quite reasonable. Although the present study cannot establish a causal relationship between uCTX-II level and pain, the results are in agreement with previous studies that have verified that uCTX-II can be used to predict knee pain in patients with knee OA [2,27]. In the same way, uCTX-II predicted 27% of the variance in WOMAC physical function score and 7% in the 40m walk test, suggesting that the higher the level of uCTX-II, the worse the self-reported physical function and the worse physical performance during a fast walk. Considering that decreased physical function is related to pain [64-66], and also increased uCTX-II level is related to increased pain, a reduction in physical function, as uCTX-II level increases, could justify the presence of knee pain. However, as we did not measure pain during 40m walk test, it is not possible to use knee pain to explain our results. Further investigation is necessary to clarify the mechanism of the influence of uCTX-II on pain and physical function in individuals with medial knee OA. Moreover, longitudinal studies would clarify the causal relationship between uCTX-II, pain, and physical function.

The present study has several limitations. We did not control for the menstrual cycle of our female participants, and postmenopausal women usually present high levels of uCTX-II [25]. However, as we used a correlation and regression analyses, subjects were analyzed using their own data. We also did not evaluate the level of physical activity [2], although it may have some influence in our findings, our subjects had limited physical

effort before the collection as urine samples were collected in the morning. In addition, considering that distinct levels of physical activity can result in different level of knee pain[67], we think that this information should be considered in future studies. The small sample size in this study may have reduced statistical power and the ability to make conclusions. Even with a small sample size, it was possible to find some statistically significant results and to provide new information regarding the relationship between cartilage metabolism and mechanical joint load. We also think that not measuring pain during 40m walk test and during the kinematic/kinetic gait assessment is a limitation, as we understand that this information would help to discuss our findings and also would help to explain participants' performance in this functional test. We only included subjects with a BMI <35kg/m² to reduce skin movement artifacts during gait analysis. Nonetheless, given that many people with knee OA are overweight or obese, these results can be partially generalized to individuals with knee OA. In the same way, as we included only subjects with predominantly medial knee OA, although it is the most affected compartment of the knee, our findings cannot be generalized to individuals with lateral and/or patellofemoral knee OA. Finally, our sample performed barefoot walking for gait analysis, we may have influenced our results as recent studies have shown reduced peak ground reaction forces during barefoot walking when compared to shod conditions [68,69].

In conclusion, greater uCTX-II level is associated with higher pain and reduced physical function and 40m walk test performance in individuals with medial knee OA.

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538 Table 1. Demographic and subject gait characteristics.

	KOA group (n=25)
Female (n, %)	12 (48)
Age (years)	58.2 ± 4.7
Height (m)	1.7 ± 0.1
Mass (kg)	79.5 ± 13.6
BMI (kg/m^2)	28.4 ± 3.9
WOMAC Score	
Pain (0-20)	8.2 ± 3.8
Stiffness (0-8)	3.4 ± 1.9
Physical Function (0-68)	24.0 ± 13.5
Walk test – 40m (m/s)	1.7 ± 0.3
Severity (KL)	
Grade 2 (n, %)	15 (60)
Grade 3 (n, %)	10 (40)
Gait speed (m/s)	1.18 ± 0.16
uCTX-II (ng/mmol crea)	26.6 ± 14.9
Peak KAM (Nm/kg.Ht)	3.02 ± 0.82
Peak KFM (Nm/kg.Ht)	2.56 ± 1.48
KAAI (Nm/kg.s.Ht)	1.19 ± 0.46

539 Data are mean ± standard deviation or frequency (proportion).
540 KOA: knee osteoarthritis, BMI: body mass index, WOMAC: Western Ontario &
541 McMaster Universities Osteoarthritis Index, KL: Kellgren and Lawrence classification,
542 uCTX-II: urinary C-Telopeptide fragments of type II collagen, ng: nanogram, mmol:
543 millimole, crea: creatinine, Nm: newton meter, Ht: height, KAM: knee adduction
544 moment, KFM: knee flexion moment, KAAI: knee adduction angular impulse.
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Table 2. Pearson correlation coefficient (r) between uCTX-II level, knee moments, symptoms, gait speed, age, BMI and physical function.

	uCTX-II Level	**p-value**
	r	
WOMAC Pain score	0.49 *	0.04
WOMAC Physical Function score	0.53 *	0.02
Walk test (40m)	-0.48 *	0.04
Peak KAM (Nm/kg.Ht)	-0.04	0.89
Peak KFM (Nm/kg.Ht)	0.03	0.55
KAAI (Nm/kg.s.Ht)	0.14	0.90
Gait speed (m/s)	-0.54*	0.03
Age (years)	0.37	0.10
BMI (kg/m^2)	0.17	0.75

*Significant correlation ($p<0.05$).

uCTX-II: urinary C-Telopeptide fragments of type II collagen, WOMAC: Western Ontario & McMaster Universities Osteoarthritis Index, BMI: body mass index, Nm: newton meter, Ht: height, KAM: knee adduction moment, KFM: knee flexion moment, KAAI: knee adduction angular impulse.

582 Table 3. Hierarchical Linear Regression Predicting pain and physical function.

Dependent variable	Step	Independent variable	R	R^2	ΔR^2	p-value
WOMAC Pain score	1	Severity and BMI	0.59	0.35*	0.35	0.04
	2	uCTX-II	0.67	0.44*	0.09	0.04
WOMAC Physical Function Score	1	Severity and BMI	0.43	0.19	0.19	0.21
	2	uCTX-II	0.67	0.45*	0.27	0.03
Walk test (40m)	1	Severity and BMI	0.62	0.39*	0.39	0.02
	2	uCTX-II	0.68	0.46*	0.07	0.03

583 *Significant difference ($p<0.05$)

584 WOMAC: Western Ontario & McMaster Universities Osteoarthritis Index, BMI: body
585 mass index, uCTX-II: urinary C-Telopeptide fragments of type II collagen.
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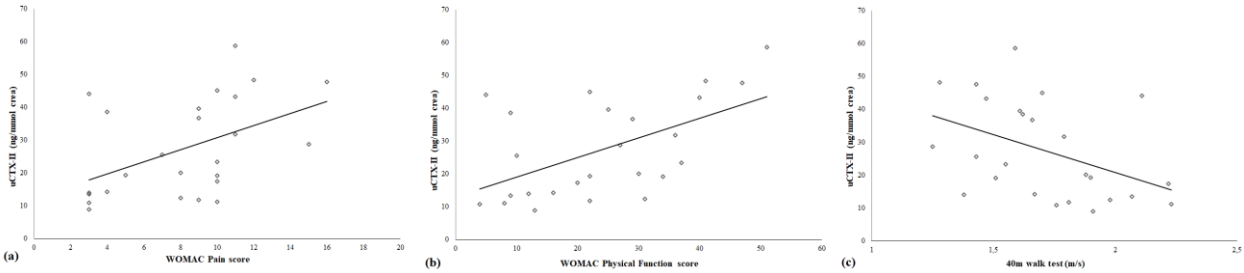
601 

602 Figure 1. Scatterplots illustrating the association between uCTX-II with WOMAC pain
603 score (a), WOMAC physical function score (b), and 40m walk test (c).
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